Bioremediation and Detoxification of Pulp and Paper Mill Effluent: A Review

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ABSTRACT

Presently, 50-60 m³ of water needed to produce a ton of paper and around 240-250 chemicals have been identified in effluents, which are produced at different stages of paper making in pulp and paper industry. The pulp and paper industry is typically associated with pollution problems related to high BOD, COD, toxicity, AOX, color, suspended solids, lignin and its derivatives and chlorinated compounds. Although numerous studies have looked ways by various researchers to remove COD, BOD, color etc. of pulp and paper effluents, the problem still persists. Number of microorganisms including bacteria, fungi and actinomycetes has been implicated to degrade the xenobiotic compounds present in pulp and paper mill effluent. This article review the origins and effects of major pollutants present in pulp and paper mill effluents and the progress made in their reduction through fungi, bacteria, algae and enzymes as well as further scope is also discussed.

Key words: Industrial wastewater, fungi, bacteria, algae, enzymes

INTRODUCTION

Industrialization improves the economic value of a nation but simultaneously it leads to the degradation of environment (Hossain and Rao, 2014; Raj et al., 2014). Economic benefits of the pulp and paper industry have led it to be one of the most important industrial sections in the world. Still now, pulp and paper mills are facing challenges with the energy efficiency mechanisms and management of the consequential pollutants, considering the environmental feedbacks and enduring legal requirements (Kamali and Khodaparast, 2015).

Generally, the pulp and paper industry has been considered to be a major consumer of natural resources (wood, water) and energy (fossil fuels, electricity) and a significant contributor of pollutant discharges to the environment. The pulp and paper industry is the sixth largest polluter (after oil, cement, leather, textile and steel industries) discharging a variety of gaseous, liquid and solid wastes into the environment. The environmental problems of pulp and paper industry are not limited by the high water consumption. Wastewater generation, solid wastes including sludge generating from wastewater treatment plants and air emissions are other problems and effective disposal and treatment approaches are essential. The significant solid wastes such as lime mud, lime slaker grits, green liquor dregs, boiler and furnace ash, scrubber sludges, wood processing residuals and wastewater treatment sludges are generated from different mills. Disposal of these solid wastes cause environmental problems because of high organic content, partitioning of chlorinated organics, pathogens, ash and trace amount of heavy metal content (Monte et al., 2009).
Environmental effects have been attributed to chemicals introduced during the manufacturing process, to natural compounds released from plant material used as mill furnish, to interactions of these compounds with each other and interactions with biota in mill effluent during waste water treatment (Hewitt et al., 2006).

The pulp and paper industry typically generates large quantities of wastewater which require proper treatment prior to discharge to the environment; otherwise it represents a significant environmental and economic problem. One significant problem is the persistent dark brown colour due to lignin and its derivatives, such as chlorolignin in the released effluent discharged from the pulp bleaching process (Prasongsuk et al., 2009). The effluent cause considerable damage to the receiving water if discharged untreated since, they have a high Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), chlorinated compounds measured as adsorbable organic halides (AOX ), suspended solids mainly fibers , fatty acids, tannins, resin acids, lignin and its derivatives, sulfur and sulfur compounds, etc. (Chandra et al., 2007). Some of these pollutants are naturally occurring wood extractives like tannins, resin acids, stilbenes and lignin. It is well established that many of these contaminants are acute or even chronic toxins. Chlorinated organic compounds like dioxins and furans have the ability to bring genetic changes in exposed organisms. This has resulted in a growing concern about the potential adverse effects of genotoxicants on aquatic biota and public health through the contamination of drinking water supplies, recreational water or edible organic species (Chandra et al., 2007, 2009).

The increasing public awareness of the fate of these pollutants and stringent regulations established by the various authorities and agencies are forcing the industry to treat effluents to the required compliance level before discharging in to the environment (D’Souza et al., 2006). Sundry studies have been conducted so far on this sector regarding the environmental impacts as well as the control of the pollutants (Singh et al., 2011). In many modern mills, reduced inputs of toxic chemicals and improved wastewater treatment have resulted in significant reduction of effluent toxicity (Van den Heuvel and Ellis, 2002) and of environmental impacts (Sandstrom and Neuman, 2003). However, most of the reviews have focused on the integrated pollution management and lack a comparative evaluation of various treatment processes particular to the water pollution control (El-Hanafy et al., 2007, 2008; Abd-Elsalam and El-Hanafy, 2009).

This article reviews the origins and effects of major pollutants present in pulp and paper mill effluents and the progress made in their reduction by using microorganisms and abiotic routes as well as further scope.

**Process description and sources of pollution:** Paper making includes five basic steps and each step can be carried out by a variety of methods. Therefore, the final effluent is a combination of waste water from each of the five different unit processes and the methods employed therein; viz. Table 1 summarizes the main pollutants, which are normally produced during several steps of pulp and paper making process (Raj et al., 2014; Karrasch et al., 2006):

- Debarking converts the plant fiber into smaller pieces called chips and removes the bark. In this step, the nature of the raw material used, i.e. hard wood, softwood, agro residues, results in the transfer of tannins, resin acids, etc. present in the bark to process water. For instance, softwoods contain a much higher quantity of resin acids than hardwoods whereas, agro residues may not contain resin acids at all.
Table 1: Major pollutants released from paper and pulp making process

<table>
<thead>
<tr>
<th>Process stages</th>
<th>Wastewater (v)</th>
<th>Pollution load</th>
<th>Effluent contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw material</td>
<td>Low</td>
<td>Low</td>
<td>Suspended solids including bark particles, fiber pigments, dirt, grit, BOD and COD</td>
</tr>
<tr>
<td>preparation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulping</td>
<td>Low</td>
<td>High</td>
<td>Color, bark particles, soluble wood materials, resin acids, fatty acids, AOX, VOCs, BOD, COD, and dissolved inorganics</td>
</tr>
<tr>
<td>Bleaching</td>
<td>High</td>
<td>High</td>
<td>Dissolved lignin, color, COD, carbohydrate, inorganic chlorines, AOX, EOX, VOCs, chlorophenols and halogenated hydrocarbons</td>
</tr>
<tr>
<td>Paper-making</td>
<td>Depends on the extent of the recycling effluents</td>
<td>Low</td>
<td>Particulate wastes, organic and inorganic compounds, COD and BOD</td>
</tr>
</tbody>
</table>


Table 2: Typical characteristics of effluents from paper and pulp production processes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>pH</th>
<th>COD</th>
<th>BOD</th>
<th>BOD/COD</th>
<th>TSS</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood yard and chipping</td>
<td>7.0</td>
<td>1275</td>
<td>556</td>
<td>-</td>
<td>7150</td>
<td>Avsar and Demirer (2008)</td>
</tr>
<tr>
<td>Thermo-mechanical pulping</td>
<td>4.0-4.2</td>
<td>3343-4250</td>
<td>-</td>
<td>-</td>
<td>320-510</td>
<td>Qu et al (2012)</td>
</tr>
<tr>
<td>Chemical thermo-mechanical pulping</td>
<td>7.43</td>
<td>7521</td>
<td>3000</td>
<td>-</td>
<td>350</td>
<td>Liu et al. (2011)</td>
</tr>
<tr>
<td>Kraft cooking section</td>
<td>13.5</td>
<td>1669.7</td>
<td>460</td>
<td>0.27</td>
<td>40</td>
<td>Wang et al. (2007)</td>
</tr>
<tr>
<td>Pulping process operations</td>
<td>5.5</td>
<td>9065</td>
<td>2440</td>
<td>-</td>
<td>1309</td>
<td>Avsar and Demirer (2008)</td>
</tr>
<tr>
<td>Bleaching</td>
<td>8.2</td>
<td>3680</td>
<td>352</td>
<td>-</td>
<td>950</td>
<td>Kansal et al. (2008)</td>
</tr>
<tr>
<td>Paper machine</td>
<td>6.5</td>
<td>1116</td>
<td>641</td>
<td>-</td>
<td>645</td>
<td>Avsar and Demirer (2008)</td>
</tr>
<tr>
<td>Integrated pulp and paper mill</td>
<td>6.5</td>
<td>3791</td>
<td>1197</td>
<td>-</td>
<td>1241</td>
<td>Avsar and Demirer (2008)</td>
</tr>
<tr>
<td>Recycled paper mill</td>
<td>6.2-7.8</td>
<td>3380-4930</td>
<td>1650-2565</td>
<td>0.488-0.52</td>
<td>1900-3138</td>
<td>Zwain et al. (2013)</td>
</tr>
</tbody>
</table>

Source: Kamali and Khodaparast, (2015), "Pulping, pulp screening, pulp washing and thickening, bleaching and kraft repulping, A combination of chlorination and alkaline extraction stages, COD: Chemical oxygen demand, BOD: Biochemical oxygen demand"

- Pulping turns the chips into pulp. This process removes the majority of lignin and hemicellulose content from the raw material, resulting in a cellulose rich pulp. Pulping can be passed out by several different methods, such as mechanical, semi chemical, kraft, sulfite pulping, etc. and once again the raw material can be utilized further (Ali and Sreekrishnan, 2001)
- Bleaching is engaged on the brown pulp obtained after pulping in order to meet the desired colour dictated by product standards. Several bleaching agents, including chlorine, chlorine dioxide, hydrogen peroxide, oxygen, ozone, etc. may be used either singly or in combination. It is in this step that lignin, phenols, resin acids, etc. get chlorinated and transformed into highly toxic xenobiotics
- Washing removes the bleaching agents from the pulp. Generally, an alkali caustic soda is used to extract color and bleaching agents from the pulp and hence, this process is also known as the alkali extraction stage (Ali and Sreekrishnan, 2001)
- Paper and paper products are finally produced by mixing the washed pulp with appropriate fillers (clay, titanium dioxide and calcium carbonate) and sizing agents like rosin and starch

The generated effluents, based on factors such as raw materials used and employed production process, commonly have a high COD (Table 2) and a low biodegradability (defined as the ratio of BOD5/COD) and more than 200-300 different organic compounds and approximately 700 organic and inorganic compounds (Karrasch et al., 2006). Such substrates may include non-biodegradable organic materials, Adsorbable organic halogens (AOX), colour, phenolic compounds, etc. (Buyukkamaci and Koken, 2010), depending upon the applied pulping process, additive chemicals and the amount of water consumed. Accordingly, in both traditional and emerging paper and pulp producers (Chen et al., 2012), such as United States (Schneider, 2011), China (Zhu et al., 2012) and India (Afroz and Singh, 2014), pulp and paper mills are considered a major source of environmental pollutants.
Hence, no single specific technology can be applied to the treatment of effluents from the two paper mills since process diversities may prevent its acceptability. So, it should be accepted in mind that each pulp and paper mill is a large, complex, highly interactive operation and that perturbations in one area may have a greater impact than expected in another area (Gutierrez et al., 2006; Hao and Man, 2006; Karrasch et al., 2006; Koschorreck et al., 2008). Subsequently, the treatment of wastewater from pulp and paper mills tends to become mill-specific and it is for this reason that the knowledge of possible contaminants present in the wastewater, their origins and degree of toxicity and available treatment technologies becomes so essential.

Environmental fate and effects of pulp and paper mill effluents: As early as the 1930's, Ebeling noted negative environmental impacts associated with pulp mill discharges (Johnston et al., 1997). Studies by van Horn in 1949, documented kraft mills as sources of compounds toxic to fish (Mishra et al., 2011). The most apparent effects on receiving water were reduced oxygen levels, eutrophication and deposition of sludges and accompanying microbial growth. Studies conducted in the early 1980's isolated chlorinated organic compounds in pulp mill effluents and receiving environments that could be traced to the use of elemental chlorine in pulp bleaching processes (Dey et al., 2013). These compounds were shown to bioaccumulate and were implicated in the toxicity and mutagenicity observed in biota in environments receiving pulp mill effluents (Ali and Sreekrishnan, 2001; Hewitt et al., 2006). Many chlorinated organic compounds have been isolated from pulp and paper mill effluents, in receiving water and in biota exposed to pulp and paper mill discharges (Dey et al., 2013; Sharma et al., 2007; Singh and Thakur, 2006). These chlorinated compounds included phenolics, fatty acids and resin acids as well as dioxins and furans, which include some of the most toxic compounds known (Ali and Sreekrishnan, 2001). In the late 1980's and early 1990's, there was increasing pressure to significantly reduce organochlorine discharges in pulp and paper mill effluents, as they were believed to be major contributors to toxicity observed in these effluents (Dey et al., 2013). Since, the 1980's process improvements replaced elemental chlorine bleaching of wood pulps in developed countries, however, elemental chlorine is still widely used in developing countries (Ali and Sreekrishnan, 2001). The removal of elemental chlorine from bleaching processes resulted in a suggested reduction in effluent toxicity and also symptomatic changes but not absence of chronic impacts (Munkittrick and Sandstrom, 1997). Pulp and paper industry is considered as one of the most polluting industry contributing 100 million kg of toxic pollutants that are being released every year in the environment (Dey et al., 2013). More than 260 chemicals have been identified present in paper and pulp mill effluents which are produced at different stages of papermaking (Hawkins et al., 2002). The toxic nature is derived from the presence of several naturally occurring and xenobiotic compounds which are formed and released during various stages of processing (Sharma et al., 2007; Singh and Thakur, 2006). The wastewater coming from paper mill containing large amount of tannins, these tend to absorb more light and heat and retain less oxygen than unprocessed water, thereby negatively affecting the aquatic flora and fauna. It was established that condensed tannins from spruce bark are toxic, not only to methanogens at concentrations present in the paper mill wastewater but also to aquatic organisms, like fish and change their behavioral response, development and growth, impact on immune system, impact on enzymes and reproductive (Dey et al., 2013; Mishra et al., 2011).

New research also demonstrated that chronic impacts previously associated with the use of elemental chlorine were observed in receiving environments downstream of mills that had never
used elemental chlorine and showed that mills that changed to Elemental Chlorine-Free (ECF) or Totally Chlorine-Free (TCF) processing still exhibited acute lethality in their discharges (Sepulveda et al., 2003). This was further complicated by the fact that there were also many process changes being implemented continuously, so effluents changed significantly over time.

Secondary treatment of effluent became mandatory in many countries and this had a huge impact on the quality of discharges. It became apparent in the 1990's that these effluents were by nature complex and variable, both temporally and spatially and that isolating toxic compounds from effluent was not going to be a simple matter (Munkittrick and Sandstrom, 1997). As, the components of effluent discharges changed, so did their environmental effects (Sharma et al., 2007; Singh and Thakur, 2006). Extrapolating data gathered from one site to what might be expected at another was problematic, partly due to the great variability of discharges and receiving environments and partly because the compounds responsible for environmental effects were not known (Hewitt et al., 2006; McMaster et al., 2006). It was observed that when acute effects disappeared, due to process changes and improvements, chronic effects emerged that had previously been masked (Munkittrick and Sandstrom, 1997).

**Advance treatment of paper and pulp mill effluent:** Ample studies have specified that pulp and paper mill effluents can potentially induce aquatic toxicity, especially at the reproductive level (Costigan et al., 2012; Hewitt et al., 2008; Waye et al., 2014). The effluent coming from the pulp and paper industry can be minimized by various internal process changes and management measures through the available technology. It was observed that near 60% of BOD of the effluent was reduced due to an internal process change in Irving Pulp and Paper Limited, Canada (Quaratino et al., 2007). A number of researchers have recommended the internal process change as a measure to control pollution (Wu et al., 2005; Yang et al., 2008; Wang et al., 2011). Singh et al. (2007) and Singhal and Thakur (2009) stated BOD, COD and color reduction by internal management measures. However, such pollutants continue to be found in the final treated effluents (Orrego et al., 2010). This is mainly due to the remaining technical problems, which lead to incomplete degradation as well as economic boundaries of some effective pulp and paper wastewater treatment methods. The study, reviewed in the present paper, have aimed to overcome such limitations of currently used pulp and paper treatments to be of cost benefit and to be able to fulfill the environmental protection requirements.

Physicochemical treatment processes include removal of suspended solids, colloidal particles, floating matters, colors and toxic compounds by conventional and non-conventional methods like sedimentation, flotation, screening, adsorption, coagulation, oxidation, ozonation, electrolysis, reverse osmosis, ultra-filtration and nano-filtration technologies, the replacement of chlorine by hypochlorite, sorption on hypo and alum-sludge, activated carbon and all phenolic compounds (Table 3) (Dey et al., 2013; Sharma et al., 2007; Singh et al., 2011).

However, these processes are quite expensive and none are considered to be commercially viable (Gutierrez et al., 2006) and still the problem remains unsolved, since lignin undergoes a spatial rather than chemical change and thus persists albeit in a different form (Singh and Kaur, 2015). Primary concerns include the use of chlorine-based bleaches and resultant toxic emission to air, water and soil with global annual growth forecast at 2-5%, the industry and its negative impacts could double by 2025 (Gutierrez et al., 2006; Wang et al., 2011).

**Biological treatment (microbes in bioremediation):** Biological treatment methods involve the utilization of microorganisms including fungi, bacteria, algae and enzymes, as a single step
Table 3: Reduction of pollution load after physicochemical treatment of paper and pulp mill effluent

<table>
<thead>
<tr>
<th>Treatment process</th>
<th>COD</th>
<th>BOD</th>
<th>DOC</th>
<th>Turbidity</th>
<th>Lignin</th>
<th>Phenol</th>
<th>Removal of pollution load (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation (aluminum chloride)</td>
<td></td>
<td></td>
<td>95.7</td>
<td>83.04</td>
<td></td>
<td></td>
<td>Wang et al. (2011)</td>
</tr>
<tr>
<td>+Flocculation (starch-g-PAM-g-PDMC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrocoagulation (Al)</td>
<td>95</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Shankar et al. (2013)</td>
</tr>
<tr>
<td>Flocculation (polydiallyldimethylammonium)</td>
<td>90</td>
<td>-</td>
<td></td>
<td>å 90</td>
<td></td>
<td></td>
<td>Razali et al. (2011)</td>
</tr>
<tr>
<td>Flocculation (chitosan)</td>
<td>80</td>
<td>-</td>
<td></td>
<td>å 85</td>
<td></td>
<td></td>
<td>Renault et al. (2009)</td>
</tr>
<tr>
<td>Flocculation (polyaluminium chloride)</td>
<td>40-45</td>
<td>-</td>
<td></td>
<td>55-60</td>
<td></td>
<td></td>
<td>Renault et al. (2009)</td>
</tr>
<tr>
<td>Precipitation (CaO)</td>
<td>80-90</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Eskelinen et al. (2010)</td>
</tr>
<tr>
<td>Electrocoagulation (CaO)</td>
<td>88</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Boroski et al. (2008)</td>
</tr>
<tr>
<td>Electrocoagulation (Al)</td>
<td></td>
<td></td>
<td>80</td>
<td></td>
<td>70</td>
<td></td>
<td>Ugurlu et al. (2008)</td>
</tr>
<tr>
<td>Adsorption (ion exchange resin)</td>
<td></td>
<td></td>
<td>70</td>
<td></td>
<td>70</td>
<td></td>
<td>Ciputra et al. (2010)</td>
</tr>
<tr>
<td>Adsorption (granular activated carbon)</td>
<td></td>
<td></td>
<td>72</td>
<td></td>
<td></td>
<td></td>
<td>Ciputra et al. (2010)</td>
</tr>
<tr>
<td>Nano-filtration</td>
<td></td>
<td></td>
<td>76</td>
<td></td>
<td></td>
<td></td>
<td>Ciputra et al. (2010)</td>
</tr>
<tr>
<td>Laccase-polymerized membrane filtration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ko and Fan (2010)</td>
</tr>
<tr>
<td>Ozonation</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M Anttari et al. (2008)</td>
</tr>
<tr>
<td>Ozonation+electrolysis</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kishimoto et al. (2010)</td>
</tr>
<tr>
<td>Solar photo-fenton (Fe2+/H2O2/UV)</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kishimoto et al. (2010)</td>
</tr>
<tr>
<td>Fung+/solar photofenton (-/H2O2)</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lucas et al. (2012)</td>
</tr>
<tr>
<td>Solar photocatalytic degradation (UV/TiO2/H2O2)</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ghaly et al. (2011)</td>
</tr>
</tbody>
</table>

COD: Chemical oxygen demand, BOD: Biochemical oxygen demand

Table 4: Cultures used for decolorization of pulp and paper mill effluents

<table>
<thead>
<tr>
<th>Microorganisms/cultures</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Algae</strong></td>
<td></td>
</tr>
<tr>
<td>Microcystis spp.</td>
<td>Iyovo et al. (2010) and Sharma et al. (2014)</td>
</tr>
<tr>
<td>Chlorella, Chlamydomonas</td>
<td>Iyovo et al. (2010), Sharma et al. (2014) and Kamali and Khodaparast (2015)</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas ovalis</td>
<td>Raj et al. (2007) and Kamali and Khodaparast (2015)</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
</tr>
<tr>
<td>Trametes versicolor</td>
<td>Dashbtan et al. (2010), Senthilkumar et al. (2014), Goksel Demir et al. (2007) and Kamali and Khodaparast (2015)</td>
</tr>
<tr>
<td>Phanerochaete chrysosporium</td>
<td>Tiku et al. (2010), Senthilkumar et al. (2014) and Kamali and Khodaparast (2015)</td>
</tr>
<tr>
<td><strong>Aspergillus niger</strong></td>
<td>Saritha et al. (2010), Senthilkumar et al. (2014) and Kamali and Khodaparast (2015)</td>
</tr>
<tr>
<td>Gloeophyllum trabeum</td>
<td>Patel and Madamwar (2002) and Gomaa et al. (2008)</td>
</tr>
<tr>
<td>Trichoderma spp.</td>
<td>Deppa et al. (2010), Senthilkumar et al. (2014) and Kamali and Khodaparast (2015)</td>
</tr>
<tr>
<td>Paecilomyces variotii</td>
<td>Senthilkumar et al. (2014), Goksel Demir et al. (2007) and Kamali and Khodaparast (2015)</td>
</tr>
<tr>
<td>Phlebia radiata</td>
<td>Aftab et al. (2011), Senthilkumar et al. (2014) and Goksel Demir et al. (2007)</td>
</tr>
<tr>
<td>Bjerkandera spp.</td>
<td>Aftab et al. (2011), Senthilkumar et al. (2014) and Goksel Demir et al. (2007)</td>
</tr>
<tr>
<td>Phanerochaete chrysosporium and T. hirsuta</td>
<td>Senthilkumar et al. (2014), Goksel Demir et al. (2007)</td>
</tr>
</tbody>
</table>

Enzymes


Treatment or in combination with other physical and/or chemical methods (Table 4) (S inhal and Thakur, 2009). Compared with physicochemical ways, biological methods for wastewater treatment are considered to be of cost benefit, eco-friendly and suitable for reduction of the BOD and COD from the effluents.
However, the conventional biological processes have not effectively performed for removal of colour and recalcitrant compounds from paper and pulp wastewater. Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less-harmful forms. So, bioremediation is employed for the treatment of various industrial effluents like paper and pulp mill effluent (Wu et al., 2005; Yang et al., 2008; Abd El-Rahim and Zaki, 2005).

**Remediation with algae:** It has been reported that some algae like *Microcystis* spp., can decolourize diluted bleach kraft mill effluents (Iyovo et al., 2010; Sharma et al., 2014). The study was observed that pure and mixed algal cultures removed up to 70% of colour within 2 months of incubation. All cultures showed a similar colour reduction pattern consisting of a phase with declining rate. The decolorization was most corrective during the first 15-20 days of incubation and then gradually declined. The decolorization by algae is caused by metabolic transformation of coloured molecules to colourless molecules with limited assimilation or degradation of molecular entities (Chandra and Singh, 2012). Recently a mixed culture of algal including *Chlorella*, *Chlamydomonas*, *Microcystis*, etc. were used for removal AOX and colour and it was reported that there was nearly a 70% AOX reduction while, color was reduced by 80% in 30 days under continuous lighting conditions. Several authors concluded though analysis of alkaline extraction of algal biomass and material balance findings indicated that the main color removal mechanism was metabolism rather than adsorption (Sharma et al., 2014; Chandra and Singh, 2012).

**Remediation with bacteria:** Number of bacterial species has been assessed for their decolorization abilities and a few of them have also been used commercially. The dominant aboriginal microbes like *Bacillus subtilis* and *Micrococcus luteus* were found competent of reducing BOD up to 87.2%, COD up to 94.7% and lignin content up to 97% after 9 days under shaking conditions and brought down pH of raw Pulp and Paper Mills Effluent (PPME) to neutral and also enhance the Dissolved Oxygen (DO) present in effluent from 0.8-6.8 mg L$^{-1}$ (Tyagi et al., 2014). It has been observed that although, numerous bacteria can decompose monomeric lignin substructure models, only a few strains are able to attack lignin derivatives obtained from different pulping processes (Hao et al., 2000; Chandra et al., 2011; Chandra and Bharagava, 2013). The cultures of *Pseudomonas aeruginosa* are capable of reducing kraft mill effluent color by 26-54% or more under aerobic conditions (Ramsay and Nguyen, 2002). Tiku et al. (2010) and Raj et al. (2007) were tested *Bacillus cereus* and two strains of *Pseudomonas aeruginosa* for the decolorization of bleach kraft effluent.

The extracellular xylanase from *Bacillus stearothermophilus* T6 is a thermostable alkali tolerant enzyme was successfully used in a large-scale bio-bleaching that bleaches pulp optimally at pH 9 and 65°C temperature. *Streptomyces badius* and *S. viridosporous* were able to use a commercial kraft lignin as sole carbon source which was characterized by Fourier transformed infra-red spectroscopy, amino acid analysis, elemental analysis for C, H, N and high performance size exclusion chromatography (Abd El-Rahim and Zaki, 2005; Chandra et al., 2011).

Two bacterial strains *Pseudomonas putida* and *Acienetobacter calcoaceticus* were studied for degradation of black liquor from a kraft pulp and paper mill in a continuous reactor. They were able to remove 70-80% of COD and lignin while, the colour removal efficiency was around 80% in 8 days (Murugesan, 2003; Abd El-Rahim and Zaki, 2005).

The degradation of dissolved and colloidal substances from Thermo-Mechanical Pulp (TMP) by bacteria isolated from a paper mill was studied. *Burkholderia cepacia* strains hydrolyzed
triglycerides to free fatty acids and liberated unsaturated fatty acids were then degraded to some extent and saturated fatty acids were not degraded. Nearby 30% of the stearyl esters, 25% of the dehydroabietic and 45% of the abietic and isopimaric resin acids were degraded during 11 days by increasing the concentration of free sterols. The degree of unsaturation seemed to be of greater importance for the degradation of fatty acids (Tiku et al., 2010; Aftab et al., 2011).

Pseudomonas, Ancylobacter and Methylubacterium strains were assessed to compare the organochlorine removal from bleached kraft pulp and paper mill effluents. These bacteria were tested for growth on chlorinated acetic acids and alcohols for adsorbable organic halogen (AOX) reduction in batch cultures of sterile bleached kraft-mill effluents from different sources. The results shown that Ancylobacter exhibited the broadest substrate range but could only effect significant AOX reduction in softwood effluents. Whereas, Melhylobacterium exhibited a limited substrate range but was capable of removing significant amounts of AOX from both hardwood and softwood effluents. By contrast, Pseudomonas spp. revealed a limited substrate range and poor to negligible reduction in AOX levels from both effluent types (Keharia and Madamwar, 2003).

Resin acids are tricyclic diterpenes that occur naturally in the resin of tree wood and bark which are transferred to process waters during pulping operations. They are weak hydrophobic acids and are considered to be willingly biodegradable and are toxic to fish at concentrations of 200-800 µg L⁻¹ in wood processing wastewater (Tiku et al., 2010; Khansorthong and Hunsom, 2009).

Apart from the investigation, mixed consortia of aerobic and anaerobic microbes for resin acid degradation, researchers have employed pure cultures of several bacteria which include Bacillus spp., E. coli, Flavobacterium spp., Pseudomonas, Acaligenes eutrophus, Anthropact, Sphinononas, Zooglea, Commanonas, Mortierella isabella, Chaetomium cochliolidae, Corticum sasaki and Fomes annosus (Tiku et al., 2010; Raj et al., 2014). Many mesophilic bacteria have been isolated and characterized for their ability to degrade resin acids. Wilson et al. (1996) isolated two species of Pseudomonas, IpA-1 and IpA-2 which were capable of growing on isopimaric acid as the sole carbon source and electron donor. These isolates were also found to grow on pimaric acid and dehydroabietic acid. A comparison of their resin acid removal capacities showed that IpA-1 and IpA-2 removed 0.56 and 0.13 µmol mg⁻¹ protein h⁻¹. Genetic relatedness of these strains was also investigated by using enterobacterial, repetitive intergenic consensus sequences to amplify genomic DNA fragments (Ramsay and Nguyen, 2002).

An alkalophilic strain of Bacillus SAM3, producing high levels of cellulose free xylanase active and stable at alkaline pH, was isolated from a soda lake. The enzyme was tested as a means of bleaching sugarcane bagasse pulp from a paper mill where the bagasse was subjected to hot alkali cooking and washing with water to neutrality. Enzymatic treatment for 2 h at pH 8 and 60°C temperature with an enzyme dose of 1.2 l U g⁻¹ pulp led to a decrease of 4 units in the kappa number. Similar treatment of the pulp at pH 7 and pH 9 indicated that the Sam-3 xylanase was effective at lowering the kappa number of the pulp over a wide pH range (Chandra and Singh, 2012).

Actinomycetes, isolated from different soil samples were tested for their ability to utilize spent sulfite bleach effluents from a paper mill. The first two bleaching stages-chlorination stage and alkaline extraction stage are monitored by determining total organic compounds and activated carbon adsorbable organic-bound halogen (AOX) in degradation and dechlorination of chlorinated compounds present in effluent. The isolates showed increased degradation rates after repeated incubations in the effluent containing medium. Separation of the culture supernatants by ultrafiltration into 3 fractions of different molecular weights revealed substantial AOX and TOC
reduction in the low molecular weight fraction. The AOX values of the higher molecular weight fractions were also reduced. Extracellular peroxidase and cell wall-bound catalase activities were produced during growth of the microorganisms on bleach effluents (Raj et al., 2007; Chandra et al., 2012).

The isolated bacteria like *Pseudolllonas putida*, *Citrobaterer* spp. and *Enterobacter* spp. can decolorized effluent up to 97% and also can reduced BOD, COD, phenolics and sulfide upto 96.63, 96.80, 96.92 and 96.67%, respectively within 24 h of growth and the heavy metals were removed up-to 82-99.80% (Keharia and Madamwar, 2003; Tiku et al., 2010).

Aerobically or anaerobically, lignin is apparently not biodegraded by rapid nor extensive bacterial. Lignin degradation has not been most extensively studied in *Streptomyces viridosporous* and *Streptomyces* but a mixed population of bacteria and protozoa derived from lake bottom sediment near the effluent kraft paper mill was shown to degrade lignin sulphonate (Raj et al., 2007).

**Factors effect on degradations processes:** The degradation of organic and inorganic load of effluent by selective bacteria depends on various factors like pH of the effluent, additional carbon source (as nutrient), agitation, inoculum size and mechanism which we used for color removal.

**Effect of pH on the color and turbidity characteristics of the effluent:** It was observed that as the pH of wastewater was dropped to as low as 2.0 because of the settling properties of the effluent are increased tremendously. This could be due to the precipitation of negatively charged lignin components and the bacterial cells due to increased protonation of the medium. Color intensity increased, as the pH was made alkaline up to 10.0. Thus, suggesting that pH plays a crucial role in the color of pulp, paper mill wastewaters (Tiku et al., 2010).

**Effect of additional carbon source:** Additional carbon source plays important role in degradation process. Number of studies established that the color removal ability increased by about 20-25% by the addition of nutrients like glucose and sucrose (Table 5). However, this could be ascribed to the hydrogen ions present in sample (pH) which lowered to less than 4.0, after 24 h. Similarly, there is negative impact in practical applicability because it shows excessive growth of biomass, leading to high turbidity in the samples. So, the use of additional nutrient sources of carbon was summarily rejected by the authors, considering the practical applicability of the technology (Tiku et al., 2010).

**Table 5:** Effect of additional carbon sources on the efficiency of *Pseudomonas aeruginosa* and Consortium 3 on decolorization of mixed inlet ETP effluent

<table>
<thead>
<tr>
<th>Sample</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent+culture</td>
<td>51±5</td>
<td>55±5</td>
<td>63±5</td>
</tr>
<tr>
<td>Effluent+culture+0.5% glucose</td>
<td>58±5</td>
<td>60±5</td>
<td>66±5</td>
</tr>
<tr>
<td>Effluent+culture+1.0% glucose</td>
<td>73±5</td>
<td>72±5</td>
<td>75±5</td>
</tr>
<tr>
<td>Effluent+culture+0.5% sucrose</td>
<td>51±5</td>
<td>60±5</td>
<td>65±5</td>
</tr>
<tr>
<td>Effluent+culture+0.75% sucrose</td>
<td>53±5</td>
<td>64±5</td>
<td>73±5</td>
</tr>
</tbody>
</table>

Source: Tiku et al. (2010) and Hao et al. (2000)
Effect of agitation and inoculum size: In decolourization studies, generally a range of 50-200 rpm was selected. It was observed that percentage of removal of colour was highest at about 150 rpm (Fig. 1), after which it became stable. Different effluent: biomass ratios of 1:1, 1:0.5, 1:0.25 and 1:2, were tried and finally the results suggested that *P. aeruginosa* (DSMZ 03504) and consortium 3 produced the best results when used in the 1:1 effluent: biomass ratio (Tiku *et al*., 2010).

Mechanism of color removal: Adsorption is playing major role to contribute the removal of color. *P. aeruginosa* was taken out from the wastewater, after the decolorization experiment and analyzed by scanning electron microscopy. Scanning electron microscopy studies showed that the bacterial cells adhering to the colored material present in the wastewater exhibited some extracellular secretion visible on the cell surfaces which was absent in the control cells. The surfaces of the cells growing in the wastewater were not as smooth as seen in the control cells. This would mean that adsorption could be a major contributor to color removal. However, the extent of adsorption was not clear and further study is required (Fig. 2) (Tiku *et al*., 2010).

Remediation with fungi: The presence of fungi are quite natural in pulp and paper mill effluent as well as sludge (Yang *et al*., 2011). They yield extracellular enzymes and can survive at higher effluent load, compared with bacteria (Singhal and Thakur, 2009; Kamali and Khodaparast, 2015). The wood degrading white-rot fungi are well efficient to degrade lignin. *Schizophyllum commune*, *Tinctoria borbonica*, *Phanerochaete chrysosporium* and *Trametes versicolor* have been found to degrade lignin and metabolize it along with carbohydrates. *Aspergillus niger* and *Trichoderma* spp., are also capable of degrading lignin and decolorizing effluent of hardwood pulp bleaching (Dashtban *et al*., 2010; Kamali and Khodaparast, 2015).

The white-rot fungus *Schizophyllum commune* can be decolourized the effluent which is coming from bagasse based pulp and paper mills. But, this *Schizophyllum commune* could not degrade lignin unless metabolizable carbon source was made available concurrently (Dashtban *et al*., 2010). It should be stated that the nutrients (carbon and nitrogen) not only improved the decolourizing efficiency of the fungus but also resulted in a reduction of BOD and COD of the effluent. Sucrose was found to be the best co-substrate for the breakdown of lignin (Frostell *et al*., 1994; Tiku *et al*., 2010; Kamali and Khodaparast, 2015).
The efficiency of treatment of effluent with *Schizophyllum commune* was highest at pH 4-5 and was further improved by intermittent aeration. *Schizophyllum commune* can be removed the colour of effluent by 90% and also reduced BOD and COD by 70 and 72% respectively under optimum conditions in 2 days incubation (Saritha *et al.*, 2010; Kamali and Khodaparast, 2015).

*Tinctoria borbonica* has been shown to decolourize the kraft waste liquor to a light yellow colour and near about 90-99% colour reduction was achieved after 4 days of cultivation (Abd El-Rahim and Zaki, 2005). According to Prasad and Joyce, *Tinctoria borbonica* also can be decolorized the hardwood effluent. It was reported that glucose was found to be the most effective carbohydrate utilized by the *Tinctoria borbonica* as, it stimulated substantial colour reduction without any increase in pollution load like COD. Under optimal conditions (pH 4.0), the total colour can remove up to 85% and COD can be decreased up to 25%, after cultivation for 3 days (Tiku *et al.*, 2010; Kamali and Khodaparast, 2015).

*Gliocladium virens* is a naturally occurring, ubiquitous soil saprophyte has been employed for the bioremediation of pulp and paper mill effluents. It was observed that the fungus grew efficiently in the effluents and can decolourized them up to 42% and also decreased the level of lignin (52%), cellulose (75%) and BOD (65%) present in effluents (Kamali and Khodaparast, 2015).
One of the most efficient lignin degrading and decolorizing white rot fungus is *Phanerochaete chrysosporium* which called as model white rot fungus because of its specialized ability to degrade the abundant aromatic polymer lignin, therefore which has been studied in much detail by Senthilkumar *et al.* (2014), Demir *et al.* (2007), Verma and Madamwar (2002) and Gomaa *et al.* (2008).

Another white rot fungus, *Coriolus versicolor*, has shown good performance. *Coriolus versicolor* produces an extracellular laccase and extracellular enzyme. Extracellular laccase plays a role in lignin biodegradation. Laccase production could not be considered as a safe indicator of lignolytic activity in the experiments because laccase did not follow any specific profile and there is no correlation could be developed between laccase production and the rate of decolourization (Aftab *et al.*, 2011; Kamali and Khodaparast, 2015). It requires a growth substrate such as cellulose or glucose for the decomposition of lignin (Tiku *et al.*, 2010). The culture conditions favouring lignin degradation are similar to those favoring fungal decolourization. The studies showed that *Coriolus versicolor* in liquid culture removed above 60% of the colour of the combined bleach kraft effluent within 6 days in the presence of sucrose. Decolourization of effluents was more efficient and friendly when the concentration of sucrose and inoculum was high (Tiku *et al.*, 2010). Sometime immobilized fungus *Coriolus versicolor*, have been used in airlift biological reactor which is made off calcium alginate beads to study the continuous decolourization (Verma and Madamwar, 2002; Kamali and Khodaparast, 2015).

The gasteromycetes *Cyprus stercoreus* which is associated with litter decomposition, can also degrade lignin as efficiently as like as other white rot fungi did (Achoka, 2002; Saritha *et al.*, 2010; Kamali and Khodaparast, 2015). The mycelial pellets, *Trametes versicolor* strain B7 oxidized the chromophores of the effluent in presence of the carbohydrates, sucrose, glucose, starch, ethanol, carboxy methyl cellulose, microcrystalline cellulose, pulp and malt extract. The highest decolourization was observed in the case of glucose at optimum condition, pH 4.5-5.5 and temperature 30°C (Diez *et al.*, 1999; Tiku *et al.*, 2010; Kamali and Khodaparast, 2015). Decolourization of paper mill effluent was studied and noticed a maximum decolourization (34%) by *Trametes versicolor* on third day in the effluent supplemented with 1.0% (w/v) glucose co-substrate and 0.2% (w/v) urea whereas, the colour reduction was observed 24% in the effluent supplemented only with glucose. It is clinched for effluent decolourization by *Trametes versicolor*, the effluent should be supplemented with glucose as a co-substrate and urea as the nitrogen source (Singhal and Thakur, 2009; Kamali and Khodaparast, 2015).

Three marine fungi like *Sordaria fimicola*, *Halosatpheia* spp. and *Basidiomycetes* spp. are able to produce the lignin modifying enzymes; laccase, manganese peroxidase (MNP) and lignin peroxidase (UP). The aptitude of these marine fungi to decolorize paper mill bleach kraft effluent was also demonstrated for the first lime (Kamali and Khodaparast, 2015).

Sumathi and Phatak (1999) were conducted number of investigation to see the capability of *Aspergillus foelidus* to remove colour, reduction of chemical oxygen demand and metabolize lignin from dissolved bagasse-based pulp and raw black and alkali-stage liquors in nutrient medium. Approximately 90-95% of the total colour was removed from growth media containing lignin at 0.05 or 0.1% or diluted black liquor or alkali liquor. Decolorization, lignolytic and COD removal processes occurred mainly during the exponential growth phase of the fungus, with associated utilization of the primary growth-supporting nutrients substrate and it proved strong correlations existed between the timing of decolorization and lignolytic processes (Sumathi and Phatak, 1999).

The degradation of cellulose by *Pleurotus sajorcaju* was rapid at the initial stages of growth. The activity of endoglucanase, exoglucanase and beta-glucosidase was maximum at 8, 12 and
26 days of growth, respectively. The activity of lignin-degrading enzymes was maximum at the later stages of growth. Such a delignification process is considered to have potential applications in the conversion of paper-mill sludge into food, animal feed and fibred products (Atkins and Clark, 2004; Ramos et al., 2009).

The optimum conditions supporting fungal growth are quite different from those favoring decolourization. The pH range for optimum growth was 4.3-4.8 and decolourization is greatly slow below pH 4 or above 5 because of poor growth. Though the fungus was grown at an optimum pH, decolourization occurs at a pH as low as 3. The decolourization is less sensitive to pH decrease than fungal growth and in the pH range of 5-7; the situation is less clear since fungal decolourization results in the formation of acids which lowers the pH rapidly (Saritha et al., 2010; Kamali and Khodaparast, 2015).

The optimum temperature of the fungus growth was 40°C, whereas decolourization takes place with a little decrease in rate at temperature as low as 25°C (Tiku et al., 2010). The optimum supporting conditions of the fungal decolourization requires sufficient oxygen and a co-substrate but the addition of nitrogen source is not necessary (Verma and Madamwar, 2002; Kamali and Khodaparast, 2015).

Remediation with enzymes: Some of the selected enzymes like ligninase, cellulase, peroxidase etc are also showing potential to remove organic load from pulp and paper mill effluents and also having vital role to improve the quality of wastewater. Out of them the most important enzymes, especially peroxidase which is used for colour removal in bleaching effluents. It is also possible to mix enzymes together with special microbes which normally do not have high enzyme activity for decolourization process. White rot fungi uses glucose as substrate and produce peroxidase, an extracellular enzymes. It seems that this enzyme oxidizes the chromophores and removes the colour from bleaching wastewater. The colour removal from effluents at neutral pH by low levels of hydrogen peroxide (H₂O₂) was enhanced by the addition of peroxidase (Raj et al., 2014; Gao et al., 2010, 2013).

Enzymes implicated in lignin breakdown in fungi (waste from wealth): Kraft lignin is a waste polymer byproduct from kraft pulping process of pulp and paper industry is disposed into the environment without considering the potential values of its derivatives causing adverse impact on natural flora, fauna as well as aquatic bodies due to dark colouration (Raj et al., 2014; Gao et al., 2010, 2011, 2013). Kraft lignin differs from natural lignin as it undergoes a variety of reactions including aryl-alkyl cleavages, strong modification of side chains and various ill-defined condensation reactions causing the polymer to fragment into smaller water alkali-soluble fragments (Chakar and Raganuskas, 2004). In spite of this, kraft lignin, though not equal to natural lignin, has been widely used as an experimental lignin for microbial degradation studies (Dashtban et al., 2010). Due their productivity, bacterial enzyme systems are expected to serve as useful tools for the bioremediation of lignin from pulping effluent and its conversion into useful intermediate metabolites.

Lignin, the most abundant aromatic biopolymer on Earth, is extremely intractable to degradation. By connecting to both hemicellulose and cellulose, it creates a barrier to any solutions or enzymes and averts the penetration of lignocellulolytic enzymes into the internal lignocellulosic structure. Some basidiomycetes white-rot fungi are capable to degrade lignin efficiently using a mixture of extracellular ligninolytic enzymes, organic acids, mediators and accessory enzymes. This review describes ligninolytic enzyme families produced by these fungi that are involved in wood
decay processes, biochemical properties, molecular structures and the mechanisms of action which condense them attractive candidates in biotechnological applications. These enzymes include phenol oxidase (laccase) and heme peroxidases [lignin peroxidase (LiP), manganese peroxidase (MnP) and Versatile Peroxidase (VP)]. Addition enzymes such as \( \text{H}_2\text{O}_2 \)-generating oxidases and degradation mechanisms of plant cell-wall components in a nonenzymatic manner by production of free hydroxyl radicals (‘OH) (Dashtban et al., 2010). These mechanisms are mostly assisted by oxidation through production of free hydroxyl radicals (‘OH) (Cong et al., 2012). Many white and brown-rot fungi have been shown to produce hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) which enters the Fenton reaction and results in release of ‘OH (Fig. 3 and 4) (Priefert et al., 1997; Landete et al., 2010). By attacking polysaccharides and lignin in plant cell walls in a non-specific manner, these radicals create a number of cleavages which facilitate the penetration of the cell wall by lignocellulolytic enzymes (Gu et al., 2011; Godoy et al., 2008). The pathways by which fungi generate free ‘OH radicals are: Cellobiose dehydrogenase (CDH) catalyzed reactions, low molecular weight peptides/quinone redox cycling and glycopeptide-catalyzed Fenton reactions (Fig. 3 and 4). Microbial enzyme systems are expected to serve as useful tools for the bioremediation of lignin from pulping effluent as well as degradation of pollution load and it convert into useful intermediate metabolites.

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**Fig. 3:** Schematic diagram of lignin degradation by basidiomycetes white-rot fungi, the major steps and enzymes involved, Lac: Laccase, LMS: Laccase-mediator system, LiP: Lignin peroxidase, MnP: Manganese peroxidase, MnP: Manganese peroxidase, VP: Versatile peroxidase, \( \text{H}_2\text{O}_2 \)-GO: \( \text{H}_2\text{O}_2 \)-generating oxidases, ASO: Aryl-alcohol oxidase, GLOX: Glyoxal oxidase, \( \text{H}_2\text{O}_2 \): Hydrogen peroxide, AAD: Aryl-alcohol dehydrogenases, QR: Quinone reductases and ‘OH: free hydroxyl radicals, Source: Bugg et al. (2011)
Fig. 4: Scheme of the fenton redox cycle found in brown-rot fungi

Fig. 5: End-of-pipe EOP treatment, which deals with the effluent discharged

RECOMMENDED TREATMENT PROCESS OF PULP AND PAPER MILL EFFLUENTS

The treatment of effluents from pulp and paper mills has become an essential prerequisite prior to their discharge to receiving water bodies. In general, remedial action taken to reduce the pollution load from pulp and paper industries is of two main types:

- Treatment at source process internal measures, wherein 'cleaner' technologies are adopted to reduce the toxicity at each stage of papermaking
- End-of-pipe EOP treatment which deals with the effluents discharged (Fig. 5)

CONCLUSION

In contrast of decolorization, different organisms show that white rot fungi particularly Phanerochaete chrysosporium, also called as model fungus and Coriolus versicolor are suitable for efficient degradation of the recalcitrant chromophoric material in bleach plant effluents. However,
the requirements for high oxygen tension and a growth substrate constrain the practical implementation of fungal decolorization. Bioremediation of pulp and paper mill effluent using fungi to decolorize effluent has yielded results similar to some of the best decolorizing activities reported in this literature. Further research is needed to develop fast biodegradation processes which are likely to provide an economically feasible process.

ACKNOWLEDGMENTS

The authors acknowledge The World Academic of Science (TWAS)- Italy and Universiti Sains Malaysia, Malaysia for financial help and for providing world class infrastructure to continue the research work.

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